RNA editing: **Rewriting receptors**

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The type of RNA editing that converts adenosine to inosine in double-stranded RNA generates different isoforms of subunits of the ionotropic glutamate-gated ion channel receptors. Recently, it has been reported that the pre-mRNA of the serotonin 2C receptor can be edited by the same mechanism.

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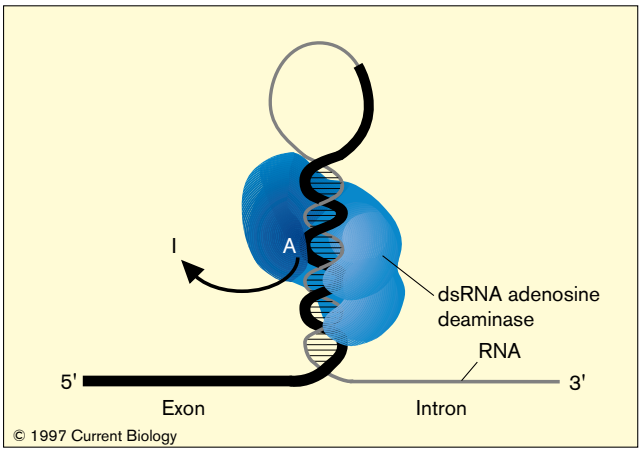
In good detective novels, the body is usually discovered first and the remainder of the novel is spent seeking the culprit. For the most part, research on RNA editing — the process by which the base sequence in RNA is altered after transcription so it differs from the DNA sequence of the gene — has faithfully followed this plot: the discovery of edited RNAs has been followed by an intense search for the enzymes responsible. But the type of RNA editing that converts adenosine to inosine in double-stranded RNA (dsRNA) has turned this plot upside down with the discovery of the enzymatic activity first, followed by the search to discover target RNAs. This search is bearing fruit, with the recent discovery that the pre-mRNA for the serotonin 2C receptor, like that for glutamate-gated channel proteins, is edited in this way.

A dsRNA-specific adenosine deaminase activity was first described in *Xenopus laevis* [1,2]. This activity, found in every metazoan tested, converts adenosine to inosine in dsRNA by hydrolytic deamination [3]. Inosine base-pairs with cytidine when cDNA is made from the edited RNA, and so the editing event will appear as a conversion of adenosine to guanosine. Unlike other RNA editing, this type does not require cofactors, guide RNAs nor specific sequence motifs surrounding the edited adenosine. Specificity is achieved by the formation of an intramolecular base-pairing interaction that folds the editing sites into a double-stranded conformation (Figure 1). In extended dsRNA, any adenosine can be deaminated while following certain preference rules [4]. This type of hypermutation has been observed in the cDNAs encoding the matrix protein of measles virus [5] and in other negative strand viruses [6]. The only other example of this type of hypermutation has been reported in *Drosophila* [7].

Unexpectedly, a family of dsRNA adenosine deaminases was found on the basis of sequence homology between

Figure 1



The dsRNA adenosine deaminases bind to the dsRNA via their dsRNA binding domains. ADAR1 contains three such domains; ADAR2 and ADAR3 both contain two. Duplex RNA is formed between exonic (black line) and intronic sequences (gray line) in the pre-mRNA for glutamate receptor channel proteins and the serotonin 2C receptor. The catalytic deaminase domain responsible for the conversion of adenosine (A) to inosine (I) is present in the carboxyl terminus of all three proteins.

different members. To date, three mammalian members of this family have been identified: dsRNA-specific adenosine deaminase (DRADA/dsRAD/ADAR1) [8], dsRNA-specific editase 1 (RED1/ADAR2) [9–13] and dsRNA-specific editase 2 (RED2/ADAR3) [14]. Even though ADAR3 has 50% sequence identity to ADAR2, it cannot convert adenosine to inosine in extended dsRNA and no target RNA has yet been found for it [14]. All three enzymes contain two or three dsRNA-binding domains in their amino-terminal region and a deaminase domain near their carboxyl terminus [8]. Homologues have been purified and cloned from other species including *X. laevis*, and other cDNAs have been suggested to encode proteins with a high degree of homology to this deaminase family [15]. Despite this, a specific correlation between enzyme and target RNA has only been made for ADAR1 and ADAR2 [9,10,12].

All the studies on the editing of specific pre-mRNAs by ADAR1 and ADAR2 have been done *in vitro*. ADAR1 is probably the most abundant protein in the family (being expressed in most mammalian tissues, as observed by northern analysis [16,17]) and was the first member of the family to be purified and cloned from different sources [8]. It was proposed that this enzyme acts on the pre-mRNA of the glutamate-gated receptor channels subunit B (GluR-B),

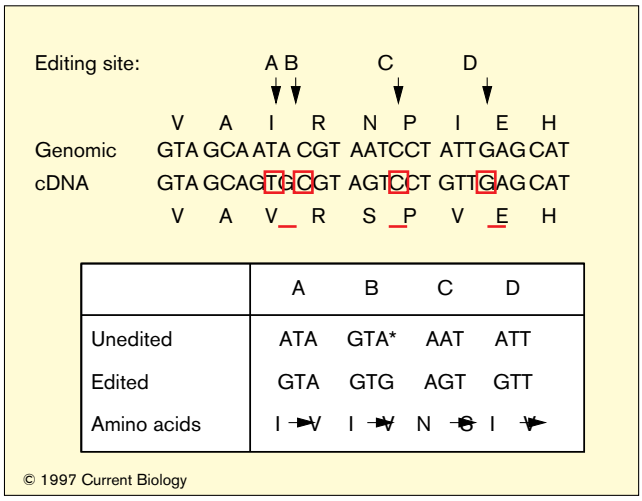
converting a glutamate (CAG) codon to an arginine (CIG) codon at what has become known as the Q/R site (Q indicating glutamate and R arginine in the single letter amino-acid code). The converted glutamate is located in the second transmembrane region, which participates in the formation of the central channel between the various subunits, through which ions can diffuse.

Conversion of the specific glutamate to an arginine renders GluR-B-containing channels impermeable to Ca^{2+} ions, with a major effect on the cell — Ca^{2+} ions being intracellular messengers that activate many cellular functions [18]. The edited GluR-B subunit is solely responsible for the impermeability to Ca^{2+} of α-amino-3-hydroxy-5-methyl-4-isoazole-propionic acid (AMPA) receptors, a subclass of glutamate receptors [19]. This RNA editing event is dependent on the maintenance of a duplex RNA structure between exonic and nearby intronic sequences [20], and inosine was shown to be incorporated into the mRNA at this edited position [21–23]. After extensive speculation that a specific cofactor allows ADAR1 to mediate this editing event, work in several laboratories eventually led to the identification of a related enzyme, ADAR2, which alone is responsible for editing the Q/R site in GluR-B pre-mRNA [9,10]. Further analysis showed that ADAR1 can edit the adenosine at the hotspot 1 site in the intron in GluR-B pre-mRNA, whereas both enzymes can edit the adenosine at the downstream R/G site [9,10].

Eight sites have been found in glutamate receptor mRNAs where specific adenosines are edited [24]. There is editing at the R/G site in the pre-mRNAs for GluR-C and GluR-D, but these have not been investigated further. The kainate receptor subunits GluR-5 and GluR-6, which are distantly related to the AMPA receptor subunits, are also edited at their Q/R site in the second transmembrane region by ADAR1 [25]. In the first transmembrane region of GluR-6, there are two edited sites termed I/V and Y/C, but it is not known which enzyme edits them [24]. It must be emphasized that it is only the adenosine at the Q/R site in GluR-B mRNA that is edited more than 99% of the time, all other sites being edited to a lesser extent. The degree of editing can also vary during development, an example being the R/G site in GluR-B pre-mRNA [26].

Recently, it has been reported that the pre-mRNAs encoding the human homologues of ADAR1 [27] and ADAR2 [11–13] are alternatively spliced. The consequence of the alternative splicing of hADAR1 is unclear, as it only has an observable effect if the dsRNA-binding domains are also mutated [27]. There are four alternatively spliced variants of hADAR2, generated by two alternative splicing events. One alternative splicing event results in differential insertion of an *Alu* cassette into the region encoding the deaminase domain of hADAR2: the

Figure 2



	A	B	C	D
Unedited	ATA	GTA*	AAT	ATT
Edited	GTA	GTG	AGT	GTT
Amino acids	I→V	I→V	N→S	I→V

The editing of the serotonin 2C receptor pre-mRNA at the four sites A, B, C and D converts the amino acids INI to VSV. The nucleotide sequence is shown above, and the changes are summarized in the table below. *Editing at site B is always accompanied by editing at site A.

forms of the enzyme that include the region encoded by the *Alu* casette have an editing activity that is two-fold lower than the shorter variants [12]. They may have an important function *in vivo*, as many sites in the GluR-B pre-mRNAs are only partially edited and the spliced variants may therefore play a role in modulating editing activity.

One major problem in the study of dsRNA adenosine deaminases has been the lack of mammalian target RNAs other than the pre-mRNAs encoding glutamate receptor subunits. Therefore, much excitement has been generated by the recent paper of Burns *et al.* [28] reporting the editing of the pre-mRNA of the serotonin 2C receptor. This is the first example of RNA editing in the pre-mRNA encoding a G-protein-coupled receptor, raising the possibility that more members of this class of receptors are edited. This editing event is a conversion of specific adenosines to inosines and it occurs at four positions, termed sites A, B, C and D, which lie close together within a 12 nucleotide duplex structure (Figure 2). The editing alters the amino acid composition in the predicted second intracellular loop of the receptor, resulting in eleven different mRNAs that encode seven major serotonin 2C isoforms (Figure 2). The fully edited version of the receptor, termed VSV, interacts with downstream G proteins 10–15-fold less efficiently than the unedited version, whereas the other isoforms appear to exhibit the same behavior as the unedited form.

The serotonin 2C pre-mRNAs are edited by ADAR1 and ADAR2. Although there are some discrepancies between the site selectivity observed by *in vitro* editing and that

seen in co-transfection experiments, it appears that ADAR1 edits sites A, B and C, while ADAR2 has the strongest preference for the D site. Analysis of serotonin 2C mRNA from different regions of the brain reveals a tissue-specific expression pattern of the different isoforms, with editing at the A, B and D sites being the most common variant found in whole brain and hippocampus. This is another example where editing by ADAR1 can vary drastically at three sites which are located within 12 nucleotides, and this fluctuation in editing particular adenosines varies in different regions of the brain. These results strongly suggest that cofactors may be involved in selecting or masking particular adenosines; alternatively, the different splice variants of the dsRNA adenosine deaminases may play a role.

The work by Burns *et al*. [28], as well as the earlier work of Seeburg and colleagues [18], has firmly established that RNA editing of pre-mRNAs encoding receptors in the brain by members of the family of dsRNA adenosine deaminases is a widespread phenomenon, generating many diverse receptor isoforms. Physiological pre-mRNA targets for RNA editing have, until now, only been found in the central nervous system. However, dsRNA-specific adenosine deaminases are present in essentially all tissues and one of the challenges of the future will be to identify additional RNA targets outside of the brain. Another important goal will be to determine the detailed molecular interaction between the different types of editing enzymes and their substrates, and to elucidate the mechanisms that control the efficiency of the reaction at different sites.

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